Project title:	Selection of strains of predatory mites that can survive applications of insecticides required for SWD control
Project number:	SF 153
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Date project completed (or expected completion date):	31 March 2017

DISCLAIMER

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GROWER SUMMARY

Headline

We have developed a strain of *Amblyseius andersoni* with increased tolerance to Tracer. This strain is not sufficiently resistant to survive field rates and further selection is ongoing.

Background and expected deliverables

The United Kingdom and the rest of Europe currently rely extensively on predatory mites for the control of mites, thrips and whitefly on crops. Of the 3,849 ha of strawberries grown in the UK in 2012, 2,567 ha were treated with *Neoseiulus cucumeris* (primarily for western flower thrips control), 2,417 ha with *Phytoseiulus persimilis* (for control of two-spotted spider mite) and 239 ha with *Neoseiulus californicus* (in protected crops) (Garthwaite *et al*, 2013). This represented a 20 fold increase since 2001. Likewise 83% of the UK raspberry production area was treated with *Phytoseiulus persimilis* in 2012 and 57% with *Neoseiulus cucumeris*.

The effective use of predatory mites relies on careful co-ordination of crop protection strategies to maintain their numbers in the crop, as predatory mites are generally considered to be more vulnerable to crop protection products than pest species. This co-ordination forms part of Integrated Pest Management (IPM) and has worked very successfully until now. However, this situation is changing with the arrival and establishment of spotted wing drosophila (SWD, *Drosophila suzukii*).

The four principal soft fruit crops grown in the UK in terms of area , strawberries (38%), blackcurrants (22%), raspberries (15%) and grapevines (15%) are all vulnerable to SWD(Cini *et al.* 2012) and outbreaks of SWD will lead to increased use of crop protection products as no IPM solution yet exists. The product groups found to offer best control of SWD around the world include the organophosphates, spinosyns and synthetic pyrethroids. Organophosphates are widely used for SWD control in the USA but their use in Europe is generally discouraged.

The arrival of SWD has created a new difficulty for growers. The necessary use of these broad spectrum control products will have adverse effects on the delicate IPM programmes which have been developed for controlling many other insect pests of soft and stone fruit crops. The answer would appear to be the development of predatory mites with resistance to such broad spectrum products and which would be commercially available to growers when required.

In this project, we aim to develop phytoseiid mites that growers can use in conjunction with spinosad and synthetic pyrethroid insecticides thus enabling them to continue using biological control programmes for spider mites and thrips.

Summary of the project and main conclusions

The predator chosen for this project was *Amblyseius andersoni* as it offers good control of spider mites and is considered a 'native' species allowing authorisation for any subsequent use on non-glasshouse crops.

Commercially available *Amblyseius andersoni* were obtained and assessed for their susceptibility to Tracer (spinosad) and Hallmark (lambda-cyhalothrin). They were found to be highly susceptible to Hallmark, but less so to Tracer and so selection commenced with Tracer. A population of selected mites was obtained and initial results indicated that tolerance of spinosad has increased.

A method was developed to rear the mites, and although this maintained populations, it was difficult to produce large scale increases in numbers. Consequently, a commercial supplier was approached to use their patented method to produce mites in larger numbers and increase the rate of selection.

This work is ongoing.

Financial benefits

Integrated pest management is of crucial importance to UK soft and stone fruit growers for pest control. The potential increased use of broad spectrum crop protection products required for SWD control will conflict with IPM programmes currently being used and this will result in rapid increases in populations of other pests such as two-spotted spider mite. The answer to this is to develop predators that are already resistant to broad spectrum products, thus allowing IPM programmes to thrive, whilst gaining control of SWD. A model for this would be the use of pyrethroid resistant *Phytoseiulus persimilis* in the Dutch chrysanthemum market, which allows spraying against capsids without loss of spider mite control (Simon Jones, Certis Europe, personal communication).

This project will maintain or improve pest control at a time of increasing pest species and populations, and reducing product availability. Results will be delivered to the industry through AHDB Horticulture. When resistant strains have been successfully developed, discussion will be held with biocontrol companies on their commercialisation.

Action points for growers

- No action points are available at this stage of the project.

SCIENCE SECTION

Introduction

Predatory mites are currently important in the control of crop pests such as spider mites, but are vulnerable to various insecticides. Whilst this can be managed by choice of insecticide, the withdrawal of some insecticides, and increased use of others to control new pest species, such as spotted wing drosophila (SWD, *Drosophila suzukii)*, will make integrated pest management (IPM) more difficult. The overall objective of this trial is to develop strains of a predatory mite resistant to spinosad and lambda cyhalothrin for use in IPM.

Choice of species is important. The choice is wide, for instance, Syngenta Bioline currently produce seven predatory mites suitable for use on strawberries or raspberries; *Amblyseius andersoni, A. barkerii, Neoseiulus californicus, N. cucumeris, A. montdorensis, Hypoaspis miles* and *Phytoseiulus persimilis*. However, regulatory restrictions on 'non-native' species, such as *N. californicus,* reduce their potential, even if these are found 'wild' on UK sites, whilst differences in ease of culture and commercial viability also apply.

The greatest threat to phytoseiid mites will come from the use of pyrethroids and spinosad. Lambda cyhalothrin is recommended for SWD control in many countries, and indeed in a trial of various insecticides in the Trento region of Italy only lambda cyhalothrin gave adequate control (Grassi *et al.*., 2012). However, pyrethroids are generally highly toxic to predatory mites (for example, Solomon *et al.* 1993, Bostanian and Belanger 1985). The toxicity of spinosad to predatory mites is unclear in the literature (Jones *et al.* 2004, Villanueva and Walgenbach, 2005, Cuthbertson *et al.* 2012), and probably varies between life stages. Given its usefulness to soft fruit growers (43% of the UK strawberry area was sprayed with spinosad in 2012, Garthwaite *et al.* 2013), it would also be valuable for growers to have spinosad compatible predators available.

Reports of small populations that have survived insecticide treatments show the potential for considerable increases in resistance. For example, natural field selection of *Typhlodromus pyri* has produced organophosphate resistant populations capable of pest control (Solomon *et al.* 1993), whilst *Amblyseius longispinosus* in China have been reported showing a 25-30 times resistance level (Zhao *et al.* 2013). Similar cases have been reported for pyrethroids, for *Amblyseius andersoni* and *Typhlodromus pyri* in French vineyards (Bonafos *et al.* 2007) and *N. californicus* in Brazilian citrus groves with, in this last case a 24 fold deltamethrin resistance ratio compared to susceptible controls (Poletti and

Omoto 2005). Careful field selection of *Typhlodromus pyri* at East Malling produced *Typhlodromus pyri* resistant to pyrethroids (Solomon & Fitzgerald, 1993),

However, naturally occurring tolerance of insecticides at these levels is comparatively rare, and very unlikely to generate sufficient numbers for bio control. Even when present, populations are diluted by immigration of susceptible mites as soon as selection pressure is eased. For reliable control growers will require a readily available source of pesticide resistant predatory mites for release into crops; generation of these mites is the aim of this project.

Materials and methods

Choice of species

Amblyseius andersoni was selected as it gives good control of spider mites and is considered a 'native' species for authorisation for any subsequent use on non-glasshouse crops. Species such as *Neoseiulus californicus*, which might have been potential candidates, and are found in UK orchards, would nonetheless have been unlikely to get regulatory approval for use, as they are officially 'non-native'.

Mite sources

Mites were purchased from a commercial supplier and used for initial trials and also subsequent selections.

Leaves were also collected from strawberries (two fields), raspberries (two fields), and apples from a total of four different farms.

Culture

Predatory mites were cultured using a modified version of the method of McMurty & Scriven (1965). Rearing arenas (Figure 1) consisted of plastic tiles on water saturated foam in plastic boxes half filled with water and detergent. Cotton wool fibres under coverslips served as shelter and oviposition sites. As a further guard against cross contamination, an adhesive (Oecotak, Oecos Ltd., Kimpton, UK) was placed along the ridge of the boxes. Any capture of mites by the adhesive was monitored and found to be minimal (data not shown).

Cultures were reared in incubators (LMS Ltd., Sevenoaks, UK) set to 20 ^{o}C, on a 16 hr light/ 8 hour dark cycle. Incubators were with air circulation, but without humidification.

Predator populations were fed with Nutrimite (Biobest, Westerlo, Belgium), a commercially available pollen source designed for feeding predatory mites.

The precise culture conditions were changed after optimisation trials as described in Culture optimisation below.

Figure 1. Mite rearing arena

Culture optimisation

An initial trial with 10 adults (five males, five females) of a commercially available population of *A. andersoni* and 0.01g of Nutrimite failed to produce any population increase. Following discussion with colleagues at EMR the population size was increased and the amount of Nutrimite decreased, as it was felt that the pollen was not being utilised and was decaying. A further four trials were conducted with two coverslips per arena, each with less than 0.001g of Nutrimite (the level of detection of the laboratory scales) and various population sizes (Table 1). Following further discussion the solid box lid, used due to lack of humidification in the incubator, was replaced with a mesh lid to reduce humidly in the box and pollen decay. This appeared to improve culture conditions.

Table 1. Optimisation of culture conditions

	Initial numbers		
Culture	**Male**	**Female**	**Notes**
1	6	7	Solid lid
2	10	20	Solid lid
3	10	20	Solid lid
4	10	20	Mesh lid

As Nutrimite has had variable results in other trials with *A. andersoni* (personal communications) the decision was taken to supplement the diet with *Tetranychus urticae* cultured on broad bean plants (*Vicia faba*).

All the above changes were implemented before insecticide trials commenced.

Large-scale culture

Because the arena method was not producing sufficient mites for further selection a commercial company (Syngenta Bioline) was approached to utilise their patented mite rearing method. This they kindly agreed to do and it is projected that greatly increased numbers should now be available. However, as their method is commercially sensitive it cannot be presented here.

Application of insecticide for bioassay

Choice of insecticide for selection was based on those recommended for SWD control in countries already with SWD infestation, which included pyrethroids, spinosad, neonicotinoids and organophosphates. Given the phasing out of neonicotinoids and the unlikelihood of authorisation for organophosphates, we focused on spinosad (in the form of Tracer, (Dow Agrosciences Ltd., Hitchin, UK), and a pyrethroid, lambda cyhalothrin, in the form of Hallmark (Syngenta UK Ltd., Cambridge, UK).

The initial proposal was to apply insecticide using a Burkard sprayer as has been used successfully on other species such as *Drosophila suzukii*. This machine passes a jet of spray droplets onto a surface below. However, it was found that the spray action of the machine blew some of the mites from the dish. Consequently, another technique was developed, using a modified method of Sato *et al.* (2000).

Briefly, papers were soaked in pesticide solution, placed in a 9cm Petri dish and left to dry. Water was used as a control and each treatment was replicated four times. Oecotak was used to ensure the mites stayed within the dish and shelters consisting of coverslips over cotton threads were provided. Mites (eight adults) were added and mortality was assessed after 24 hours, by touching the mites, with those that did not respond being counted as dead.
Initial bioassays aimed to determine a suitable dose for selection and were based on the recommended field dose. Thus lambda cyhalothrin (Hallmark) was tested at a field rate of 0.125 ml/l, 0.1x field rate (0.013 ml/l) and 0.01x field rate (0.0013 ml/l). Spinosad was tested at field rate (0.15 ml/ l) and 0.1x field rate (0.015 ml/l).

Selection for resistance

All trials used the same, commercially available, source population. The method was similar to that used for bioassays above, except that controls were in duplicate, whilst selection dishes contained variable numbers of adults and immature nymphs in order to maximise the number of mites exposed on each occasion. Selection bioassays were run on five separate occasions, exposing a total of 220 adult mites and 42 immatures. Survivors were transferred to the arenas described above for rearing.

Secondary selection of the selected population has commenced with a trial assay using the same dose of spinosad. Further trials are awaiting increases in the population.

Results

Mite culture

The initial culture methodology was modified by reduction of the amount of pollen added and improved ventilation following trials. Cultures 1, 2 and 3 failed to produce any immatures after 23, 6 and 6 days respectively, whilst Culture 4 produced immatures from Day 9 onwards. This was attributed to increased ventilation in the culture.

The diet was also supplemented with *Tetranychus urticae*.

Populations sampled from the wild

Of the five sampled fields, mites could not be found in three samples. Mites from the apples were identified as *Typhlodromus pyri*. One crop of raspberries yielded mites identified as *Amblyseius andersoni* and these were cultured as above. Two cultures were established with a total of 87 adult mites. Despite some evidence of fertility in the production of small numbers of immatures, after 121 days only four adults and one immature remained and the population was discarded shortly afterwards.

Determination of selection dose

Lambda cyhalothrin

The population was found to be susceptible to lambda cyhalothrin, with 100% mortality at Field and 0.1x Field doses (0.125 and 0.0125 ml/l). A further ten-fold dilution (0.01x Field dose, 0.00125 ml/l) gave a mortality of 76% (Figure 2).

Spinosad

The results of bioassays with spinosad are given in Figure 3. A dose equivalent to that recommended for field use, 0.15 ml/l, gave 28% mortality after 24 hours. This dose was chosen for further resistance selection trials.

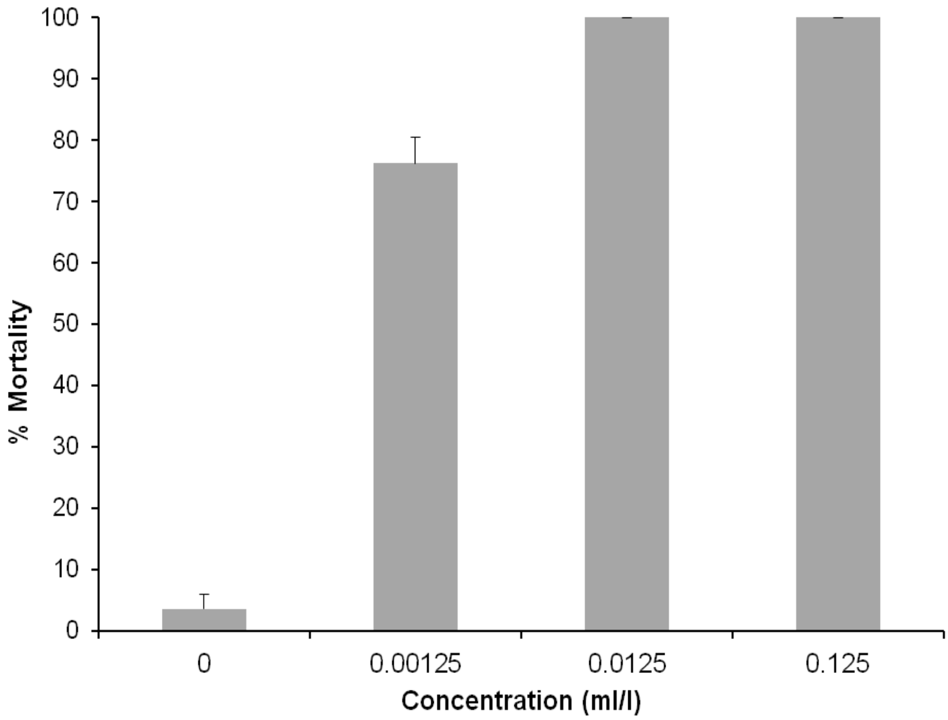

Figure 2. Mortality of *A. andersoni* adults from lambda cyhalothrin after 24 hours

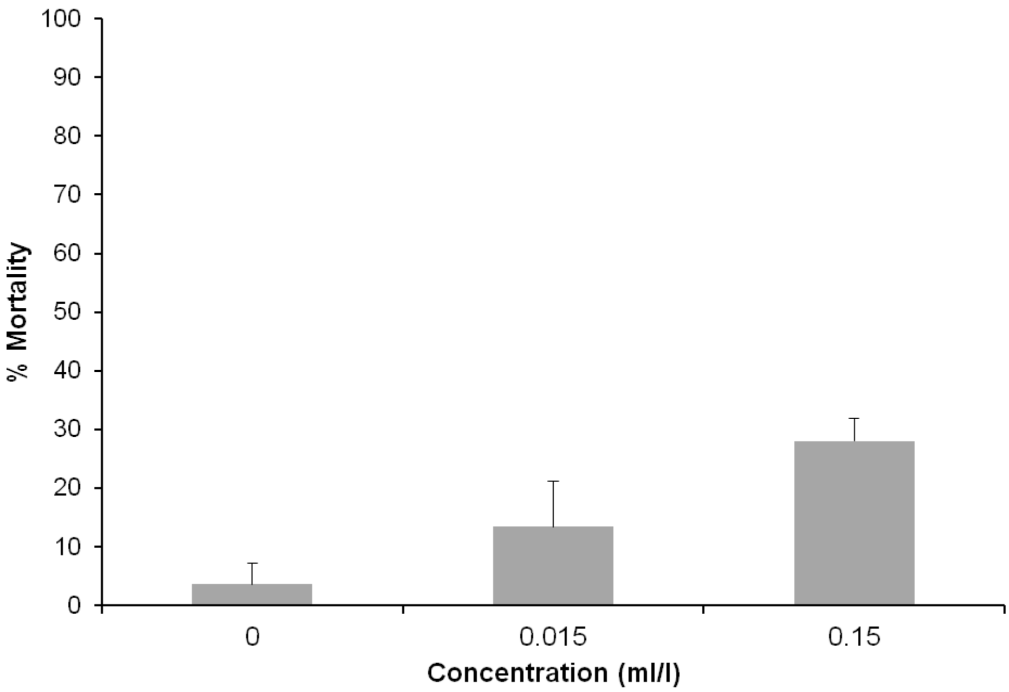

Figure 3. Mortality of *A. andersoni* adults from spinosad after 24 hours

Selection

Spinosad at a rate equivalent to field rate (0.15ml/l) was applied to a commercially available population of *A. andersoni* and the survivors were transferred to arenas for culture. The results are summarised in Table 2.

Table 2. Summary of results from selection of *A. andersoni* with spinosad

Total number used		**% Mortality**	
Adults	**Immatures**	**Adults**	**Immatures**
220	42	29	74

The % mortality for adults is similar to that found in the original trials as would be expected. However, *A. andersoni* immatures were found to be more susceptible.

A preliminary assay on the selected population has been performed (Table 3). Population numbers are currently being increased to perform further assays. Initial indications are that selection has increased the tolerance of the mites to spinosad, but this need to be confirmed with further tests.

Table 3. Summary of results from selection of *A. andersoni* with spinosad.

Total number used		**% Mortality**	
Adults	**Immatures**	**Adults**	**Immatures**
33	4	17	50

Discussion

The commercial population assessed was found to be extremely susceptible to lambda cyhalothrin, with 100% mortality at doses much lower than those likely to be found in the field. This is in agreement with trials on phytoseiid mites in the literature (for example, Solomon *et al.* 1993, Bostanian & Belanger 1985).

In contrast, spinosad killed an average of 28% of adult mites at field rates. It was therefore felt that selection with spinosad offered a more likely route to successful selection in the short to medium term. The dose selected for selection was that equivalent to the field rate as this was both sufficiently toxic to remove susceptible individuals from the population and was relevant to the natural situation.

Culture of mites was more difficult than expected, and although changes in the protocol meant that populations could be maintained, the population increase was minimal and did not provide sufficient mites for large scale selection. However, rearing of populations by a by a commercial company looks likely to remedy this problem.

Both adults and immatures were exposed to the selecting dose. On average, 29% of adults were killed, the survivors being cultured. In contrast 74% of immatures were killed. This differential effect on different life history stages has been found with other insecticides (for example, Kaplan *et al.* 2012).

Preliminary data suggest that section with spinosad should be successful with higher survival under the same discriminating dose of the previously selected population. This work is ongoing.

Conclusions

- Tracer (spinosad) and Hallmark (lambda cyhalothrin) were used to assess the resistance status of a commercially available population of *Amblyseius andersoni*.
- Mites were found to be highly susceptible to Hallmark, but less so to Tracer. A population of Tracer selected mites was obtained and initial results indicated that tolerance to spinosad has increased.
- A method was developed to rear the mites, and although this maintained populations it was difficult to produce large scale increases in numbers. A commercial supplier was approached to use their patented method to produce mites in larger numbers and increase the rate of selection.
- Further Tracer selection is ongoing

Knowledge and Technology Transfer

A summary of the project and initial results was presented at the AHDB Horticulture meeting on 26 November 2014.

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